

REMARKS**Claim Amendments**

New claim 79 is provided herewith, and claims 44, 60, 77, and 78 are amended herewith, without disclaimer of subject matter and solely to advance prosecution. These amendments present rejected claims in better form for allowance or consideration on appeal and accordingly Applicants respectfully request their entry. As was discussed in a telephonic interview, these amendments obviate several of the rejections. Specifically, the amendment of claims 44, 60, and 78 to recite “chymosin from a bovine *or Camelidae species or Camelus dromedarius*” conforms to the scope indicated to have adequate written description support in the Office Action. Support for this amendment is found in at least original claim 29. Similarly, the amendment to claim 77 to recite “wherein the glucoamylase is derived from *culture of an Aspergillus species*” eliminates susceptibility to the interpretation that the Examiner adopted in making the rejections. Support for this amendment is found in at least original claim 26.

Additionally, claim 44 is amended to remove the recitation “lactic acid” from the Markush group and instead present it in a separate independent claim (which is new claim 79 in this paper). As discussed in the telephonic interview, the presentation of lactic acid in a separate independent claim is intended to place the application in better condition for allowance or appeal because lactic acid raises different issues owing to its lower pK_a than acetic acid. Support for this claim is found as stated previously with claim 44 (see Applicant’s remarks dated July 20, 2010, pg. 6). Applicants further note that new claim 79 does not raise any additional issues that would preclude its entry because lactic acid was previously recited in claim 44 and has been particularly addressed in the prior office action.

Since these amendments place the claims in better condition for appeal and do not raise new issues, their entry after a Final Rejection is permissible. *See* MPEP 714.12 and 37 C.F.R. § 1.116(b)(2). Accordingly Applicants respectfully request entry of these amendment.

No new matter has been added by these amendments.

Interview Summary

Applicants are grateful for the telephonic interview conducted on October 14, 2010. Present on the call were Examiner Steadman and Applicant’s representatives Robin Teskin and

Ken Kalafus. During the interview, Applicants described proposed claim amendments intended to obviate the majority of the issues in the application. In claims 44, 60, and 78, Applicants proposed amending the claims to recite “a gene encoding chymosin from a bovine ~~or Camelidae species or Camelus dromedarius~~” which had been indicated in the Office Action to have sufficient written description support. Additionally, Applicants proposed to amend claim 77 to recite “wherein the glucoamylase is derived from culture of an *Aspergillus* species,” which is not susceptible to the interpretation that the Examiner adopted in making the written description, enablement, and new matter rejections. These amendments are reflected in the listing of the claims provided herewith.

The remaining issue, obviousness, was also discussed. Central to each rejection is Lawlis reference (U.S. Patent No. 5,801,034). Lawlis discloses a method of killing cells by adding an organic acid and lowering the pH of the medium. The pH used in the Lawlis method differs depending on the particular organic acid chosen, and the rejections have been differently stated depending on the acid involved (acetic acid, lactic acid, and formic acid). Because these three acids raise different issues, Applicants proposed to remove the recitation “lactic acid” from the Markush group in claim 44 and instead present it in a separate independent claim (which is new claim 79 in this paper). Applicants explained that these amendments amendment would place the application in better condition for allowance or appeal because the obviousness rejection of each independent claim would only relate to a single organic acid: acetic acid (claims 44 and 78), formic acid (claim 60), or lactic acid (new claim 79). Applicants then described their arguments traversing the obviousness rejections, which are presented below.

Response to rejections concerning Acetic Acid

Claims 44-46, 50-55, 58-59 and 78 have been rejected as allegedly obvious over Lawlis in view of Ward (Office Action, page 26), and claim 57 has been rejected over these references and in further view of EMBL AJ131677 (Office Action, page 30). Lawlis teaches a method of killing cells without lysis. The Lawlis method simplifies recovery and purification of secreted enzymes because the intact cells are easier to remove from the media than the cytoplasmic contents that would be released from lysed cells. It is undisputed that Lawlis only teaches a method of killing cells, and does not provide any guidance for selection of pH values that would achieve the beneficial result recited in the present claims, namely inactivating unwanted

glucoamylase activity while maintaining chymosin activity. Notwithstanding these differences, the Examiner has alleged that it would have been obvious to use the method of Lawlis, with acetic acid, and within the range of pH values recited in the present claims, to kill the cells taught by Ward. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Though Lawlis does claim an open-ended range of pH values, the reference does not justify lowering the pH to between 1.8 and 1.0 as recited in claims 44 and 78 (or any of the sub-ranges that are recited in the dependent claims). Rather, Lawlis demonstrates complete cell killing with acetic acid at pH higher than the values recited in the claims. Specifically, in examples 1 and 2, no viable cells remained after treatment with acetic acid at pH 2.0. Because Lawlis only teaches using acidic treatment to kill cells and this goal is completely achieved with acetic acid at pH 2.0, Lawlis does not justify lowering the pH to between 1.8 and 1.0 as recited in claims 44 and 78.

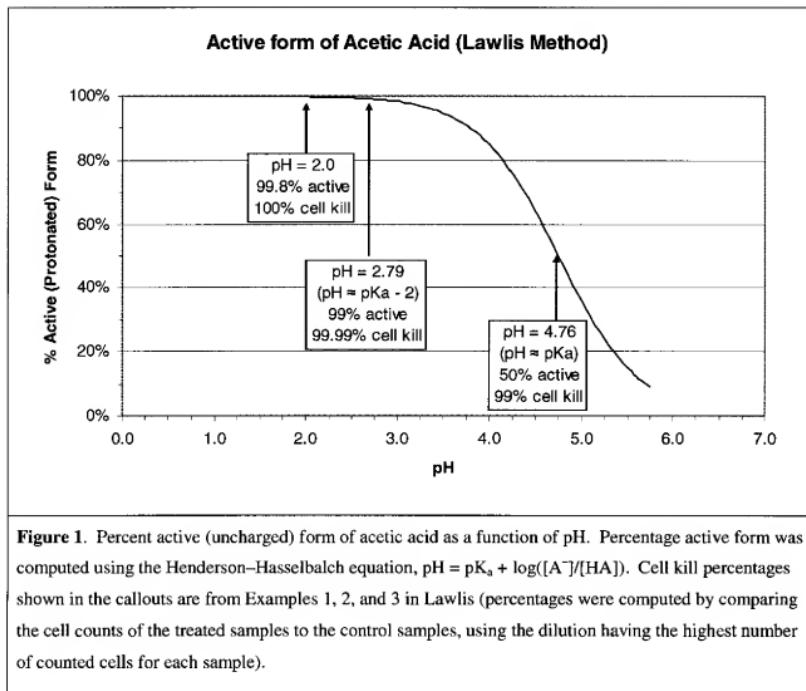
The mechanism of cell killing also does not provide any justification for lowering the pH to within the claimed range. Rather, the mechanism of action indicates that further lowering of pH would be inconsequential. That mechanism of action is as follows:

By reducing the pH of the mixture or media to a value equal to or less than two pH units below the pK_a of the organic acid to be used, the acid is 99% protonated or uncharged and becomes "invisible" to the cell as an acid.

The cell may then take up or import the neutral acid compound in the usual manner as a nutrient. . . . which kills the cell.

Lawlis, col. 4, lines 13-21. Based on this mechanism of action the Examiner has alleged that Lawlis teaches that pH is a result-effective variable for achieving cell kill because the percentage of active form could be slightly increased with lowered pH (Office Action, page 27) and has concluded that one of ordinary skill in the art would have been motivated to lower the pH farther than 2 units below the pK_a to achieve a more complete cell kill. *However, Lawlis teaches that organic acid concentration, rather than pH, is the critical result-effective variable* once the pH has been lowered to $pK_a - 2$. This is because once the pH is lowered to $pK_a - 2$, the organic acid is already 99% in the active form. Even the most extreme lowering of pH (e.g., adding an excess

volume of fuming hydrochloric acid) could only increase the active acid concentration by about 1%. In contrast the active acid concentration can readily be doubled, tripled, or even further increased (relative to the concentrations in the working examples) by simply increasing the organic acid concentration, because the active acid concentration increases proportionately to the total organic acid concentration. Thus, once the $pK_a - 2$ threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase cell killing by lowering the pH below $pK_a - 2$ because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.



The insignificance of lowering the pH below $pK_a - 2$ is illustrated in Figure 1 above. As pH is lowered to 2.75 (two units below pK_a), the percentage of active form rapidly increases to

99%. Below this point, the curve flattens and the percentage of active form asymptotically approaches 100%. Though there may be some minor increase in the amount of acetic acid in the active form, Lawlis teaches that 99% active form is highly effective at cell killing and that further lowering of pH would have insignificant impact. For example, at pH 2.79 (*i.e.*, approximately equal to $pK_a - 2$), 99% of the acetic acid is in the active form, and 99.99% of the cells were killed in four hours (Example 3, Sample # 6). Though a longer overnight treatment at pH 2.0 (99.8% of acetic acid in active form) achieved 100% cell kill (examples 1 and 2), Lawlis does not suggest any preference for the pH 2.0 treatment, but apparently treats the additional 0.01% cell kill as either mathematically insignificant or attributable to the greater treatment duration (overnight instead of 4 hours). Thus, Lawlis does not express any preference for a pH lower than $pK_a - 2$, and both the empirical cell killing results and the mechanism of action do not suggest any benefit to lowering the pH below $pK_a - 2$.

The express teachings of Lawlis also do not support the Examiner's contention that it would have been obvious to lower the pH below $pK_a - 2$ to achieve greater cell killing. For example, Lawlis nowhere states that there would be any benefit to lowering pH below $pK_a - 2$. Rather, Lawlis only teaches that greater cell killing can be achieved with greater organic acid concentration. *See* Lawlis, col. 3, lines 38-45 (describing exemplary acetic acid concentrations sufficient to achieve cell killing); col. 3, lines 62-64 ("After the pH is adjusted to the proper level, the organic acid is added in an amount sufficient to effect the desired cell kill.") Moreover, increasing the organic acid concentration does not affect the pH in the Lawlis method; rather, the target pH is achieved by decreasing the amount of inorganic acid to offset any increase in organic acid concentration, or alternatively Lawlis teaches adding an organic acid salt to achieve the same effect as an organic acid without altering pH. *See* col. 3, line 65 to col. 4, line 9, col. 4, lines 28-38, and Example 3.

The Examiner has further alleged that one of ordinary skill in the art would have found it obvious to use a pH value of 1.75 because this pH value is mentioned in Lawlis and would recognize that this pH value is below $pK_a - 2$. Office Action, page 27. However, this pH value is only recited for use with formic acid, whose pK_a is 3.75.

the pH of the culture or fermentation mixture is first adjusted using a mineral acid to a pH approximately equal to or less than about 2 pH units

below the pK_a of the organic acid selected for use for the cell kill. For example, if formic acid ($pK_a=3.75$) is to be used to accomplish the cell kill, the pH of the mixture will be adjusted with a mineral acid to about 1.75 or less

Lawlis, col. 3, lines 56-60 (emphasis added). This is the only mention of pH 1.75 (or any pH value below 2.0) in the entire reference, and Lawlis explicitly states that this pH value is only chosen for formic acid because it is 2 pH units below the pK_a of that particular acid. Thus, contrary to the alleged basis of rejection, Lawlis does not teach use of pH 1.75 for any organic acid other than formic acid, but rather teaches that the pH value is to be selected based on the pK_a value of the organic acid. Formic acid has a different pK_a than acetic acid, and therefore the pH taught for formic acid is immaterial. Rather, since the pK_a of acetic acid is 4.75, the pH value taught for that acid is 2.75 ($pK_a - 2$).

Thus, where acetic acid is used (pK_a 4.75), Lawlis does not provide any justification for lowering the pH to any value lower than 2.75 (i.e., $pK_a - 2$). If greater cell killing is desired, Lawlis teaches increasing the acid concentration, but nowhere suggests any benefit to lowering the pH any lower than 2.75 when using acetic acid. The mechanism of cell killing also does not provide any justification for further lowering of the pH, since the critical result-effective variable is organic acid concentration once the pH has been lowered to $pK_a - 2$, and further lowering of pH is inconsequential. The working examples also illustrate complete cell kill at pH values higher than between 1.8 and 1.0 as recited in the claims; since the purpose of the Lawlis method (killing cells) is completely achieved at these pH values, there is no rationale for lowering the pH any further. Finally, the secondary references fail to remedy these deficiencies of Lawlis. Therefore, there is no justification for lowering the pH to any value between 1.8 and 1.0 when using acetic acid with the Lawlis method, and accordingly claims 44-46, 50-55, 57-59 and 78 are not obvious.

The pK_a of lactic acid is 3.86

The prior Office Action included an obviousness rejection based on the use of lactic acid with the Lawlis method. As discussed above, Lawlis teaches selecting the treatment pH based on the pK_a of the organic acid, thus the pK_a of lactic acid is of fundamental importance to the rejection. This pK_a value has been disputed during prosecution. Applicants have previously

provided an authoritative source (the 2006 Merck Index) showing the pK_a value of lactic acid to be 3.86. The Examiner had identified one patent (Van Ooijen, U.S. Patent No. 5,371,287) and now has identified one non-patent literature publication (Griffin *et al.*, Substrate-dependent proton load and recovery of stunned hearts during pyruvate dehydrogenase stimulation. *Am J Physiol Heart Circ Physiol.* 2000 Jul;279(1):H361-7) which state that the pK_a of lactic acid is 3.08. In weighing which reference teachings to adopt, the Examiner gave three reasons to reject the teachings of the 2006 Merck Index. First, the Examiner stated that the 2006 Merck Index indicated pK rather than pK_a values and did not indicate whether these were equivalent. Office Action, page 24. To address this concern, Applicants present Exhibit A which provides pages of the CRC Handbook of Chemistry and Physics (2003-2004 edition) which shows the pK_a of lactic acid is 3.86. Because this pK_a value is exactly the same as the “pK” value for DL-lactic acid in the 2006 Merck Index, it is apparent that these terms refer to the same constant.

Second, the Examiner has stated that because it was published after the present application’s filing date, the 2006 Merck Index was “not relevant to establishing the known pK_a of lactic acid *at the time of invention.*” Office Action, page 23 (emphasis in original). However, Applicants respectfully submit that the methods of measuring pK_a are firmly established in the art, such that it would be inconceivable for the accepted pK_a value to change so dramatically in just a few years, and thus a pK_a value reported in 2006 is representative of the contemporaneous understanding in the art on the date that the present application was filed. Nonetheless, to advance prosecution, Applicants present herewith Exhibit B which provides pages of the 1989 Merck Index providing the pK_a for DL-lactic acid is 3.86: the reference shows the equilibrium constant, K is 1.38×10^4 and states that $pK = 1 / \log_{10} K$ (*see* definitions of K and pK at pgs. xvi and xvii), which is 3.86. As noted above, the pK value for lactic acid furnished in the Merck Index is the same as the pK_a value reported in another authoritative source, the CRC Handbook of Chemistry and Physics, and accordingly the K value reported in the 1989 Merck Index demonstrates that the pK_a of lactic acid was known to be 3.86 prior to the filing date of the present application.

Third, the Examiner has alleged that the discrepancy between pK_a values between the 2006 Merck Index and the two references identified by the Examiner suggests controversy in the art concerning the pK_a value of lactic acid, and that in the face of such controversy one would adopt the lower pK_a value. Office Action, page 24. However, nothing in the references

acknowledges the existence of any such controversy or suggests that the discrepancy could result from anything other than repetition of a typographical error. In the face of a discrepancy one would naturally turn to an authoritative source for resolution. Applicants believe that the Merck Index and CRC Handbook of Chemistry and Physics are so well-known as authoritative sources of chemical data that no evidence of should be required to show that they are regarded as authoritative. Nonetheless, to advance prosecution Applicants present excerpts from editorial reviews to show that these publications have long been established as authoritative sources in the area of chemical data.

Excerpts from editorial reviews of the Merck Index are as follows:

"The Merck Index was first published in 1899, and it will continue to serve as the standard reference for chemists, biochemists, pharmacologists, pharmacists, and other health professionals." (Journal of Medicinal Chemistry, February 8, 2007)

"...the quality of the contents in one concise volume makes TMI the premier work of its kind..should be available as part of the arms-reach searching armamentarium of laboratory scientists of many stripes..." (Journal of Chemical Information and Modeling, March 2007)

"Scientists working in many different areas...will be looking forwards to the future editions with their continued tradition of excellence." (American Journal of Therapeutics, September/October 2007)

"...a must for academic, public, and special libraries...essential."
(CHOICE, June 2007)

See <http://www.amazon.com/Merck-Index-Encyclopedia-Chemicals-Biologicals/dp/091191000X> (retrieved October 20, 2010).

Excerpts from editorial reviews of the CRC Handbook of Chemistry and Physics are as follows:

This famous handbook continues to provide current, critically evaluated chemical and physical data in a one-volume format. A goldmine of information....(JACS, Vol. 127, No. 12, 2005)

A standard text in libraries everywhere...this resource is an invaluable source of data. (Medical Reference Services Quarterly, Vol. 24, No. 3, Fall 2005)

The CRC Handbook has been, in successive editions, on my bedside table for the last 55 years. (Oliver Sacks, Neurologist and author)

This well-established publication from CRC Press has been given a face lift The publisher's philosophy, however, 'to provide broad coverage of data commonly encountered by physical scientists and engineers,' remains unchanged. (Prof. John Yates, Chemical Engineering Research and Design, May 2006)

See <http://www.amazon.com/CRC-Handbook-Chemistry-Physics-88th/dp/0849304881> (retrieved October 20, 2010). Thus, it is clear that the Merck Index and the CRC Handbook of Chemistry and Physics are well accepted as authoritative sources of chemical data such as pK_a values. Because these authoritative sources are in agreement that the pK_a value for lactic acid is 3.86 and there is no evidence of any scientific controversy in the field concerning its value, one of ordinary skill in the art would have adopted this value and have disregarded the values reported in the Griffin and Van Ooijen references as plainly erroneous.

Response to rejections concerning Lactic Acid

Due to their recitation of lactic acid, claims 44-46, 50-51, 55, and 58-59 were previously rejected as allegedly obvious over Lawlis in view of Ward, Chang, and Van Ooijen (Office Action, page 23, and claim 57 was rejected in further view of Wangoh and EMBL AJ131677 (Office Action, page 25). The present amendment removes the recitation "lactic acid" from the Markush group in claim 44 and instead presents it in new **claim 79** and accordingly the rejections are now discussed with reference to this claim. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Lawlis does not expressly mention lactic acid but does recite using the method with organic acids having between 1 and 5 carbon atoms (see, e.g., Lawlis, claim 1). The Examiner

has alleged that one of ordinary skill in the art would have found it obvious to use lactic acid (which has 3 carbons) with the Lawlis method of killing cells, and within the range of pH values recited in the claim 44, to kill the cells of Ward. Specifically, because the Examiner had concluded that the pK_a of lactic acid was understood in the art to have been 3.08 at the time of filing, the rejection was based on an allegation that one of ordinary skill in the art would have found it obvious to use lactic acid with the Lawlis method at a pH of 1.08. However, Applicants have presented ample evidence that the pK_a of lactic acid was and still is accepted to be 3.86, notwithstanding typographical errors in the two non-authoritative sources that were identified by the Examiner. Accordingly, the pH value that would be used for lactic acid with the Lawlis method would have been 1.86, rather than 1.08 as alleged by the Examiner. This value is above the claimed pH range (which is between 1.7 and 1.0 in claim 79).

The Examiner has further alleged that one of ordinary skill in the art would have found it obvious to lower the pH to below pK_a - 2 to achieve greater cell kill. Office Action, page 24. However, as discussed above with acetic acid, Lawlis teaches that the pH value need only be lowered to pK_a - 2, *i.e.*, 1.86 for lactic acid, and further lowering of pH below this value is inconsequential. If greater cell killing is desired, Lawlis teaches that organic acid concentration is the critical result-effective value once the pH has been lowered to pK_a - 2. Once this pH value has been reached, 99% of the organic acid is in the active form, such that even the most extreme further lowering of pH could only increase the active acid concentration by about 1%. In contrast, active acid concentration increases proportionately to total organic acid concentration, and accordingly the active acid concentration can readily be doubled, tripled, or even further increased by simply increasing the organic acid concentration. Thus, once the pK_a - 2 threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase cell killing by lowering the pH below pK_a - 2 because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.

Because acid concentration (and not pH) is the result-effective variable once the pH has been lowered to pK_a - 2, Lawlis does not provide any justification for lowering the pH any lower than 1.86 when using lactic acid. Moreover, Lawlis only teaches a method of killing cells and does not provide any guidance for selection of a pH value to achieve the beneficial result recited

in the present claims (inactivating unwanted glucoamylase activity while maintaining chymosin activity). The secondary references fail to remedy this deficiency of Lawlis. Therefore, there is no justification for lowering the pH to between 1.7 and 1.0 when using lactic acid with the Lawlis method, and accordingly claim 79 is not obvious.

Response to rejections concerning Formic Acid

Claims 60-62, 66-71, and 74-77 have been rejected as allegedly obvious over Lawlis in view of Ward (Office Action, page 18), and claim 73 has been rejected in further view of EMBL AJ131677 and Wangoh (Office Action, page 18). Though the rejected claims recite an inorganic acid, the Examiner has stated that these claims use an open transitional phrase and therefore are open to the presence of formic acid in addition to the inorganic acid. Based on this interpretation, the Examiner has alleged that it would have been obvious to use the method of Lawlis, with formic acid, and within the range of pH values recited in the present claims, to kill the cells taught by Ward. Applicants respectfully traverse and request reconsideration and withdrawal of the rejection for the reasons stated in their previous response and for the further reasons described below.

Lawlis teaches that the pK_a of formic acid is 3.75 and that cell killing is accomplished by lowering the pH to $pK_a - 2$. The Examiner has alleged that since Lawlis teaches using pH 1.75 with formic acid, one of ordinary skill in the art would have found it obvious "to adjust the pH of a medium with a mineral acid to 1.7 using only routine experimentation." Office Action, page 15. However, routine experimentation must have a purpose. Lawlis only teaches a method of killing cells, and this purpose does not justify lowering the pH below 1.75 with formic acid. Rather, if greater cell killing is desired, Lawlis teaches that organic acid concentration is the critical result-effective value once the pH has been lowered to $pK_a - 2$. Once this pH value has been reached, 99% of the organic acid is in the active form, such that even the most extreme further lowering of pH could only increase the active acid concentration by about 1%. In contrast, active acid concentration increases proportionately to total organic acid concentration, and accordingly the active acid concentration can readily be doubled, tripled, or even further increased by simply increasing the organic acid concentration. Thus, once the $pK_a - 2$ threshold has been reached, the organic acid concentration is the critical result-effective variable. Accordingly, contrary to the alleged basis of rejection, one would not be motivated to increase

cell killing by lowering the pH below pK_a - 2 because this would be ineffective; rather, if greater cell killing were desired then the organic acid concentration would be increased without altering pH.

For the foregoing reasons, Lawlis does not provide any justification for lowering the pH any lower than 1.75 when using formic acid. The secondary references fail to remedy this deficiency of Lawlis. Therefore, there is no justification for lowering the pH to between 1.7 and 1.0 when using formic acid with the Lawlis method, and accordingly claim 60 is not obvious. The remaining claims all properly depend from claim 60 and accordingly are not obvious for at least the same reasons.

CONCLUSIONS

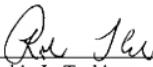
Applicant submits that these amendments and arguments overcome all of the rejections as stated in the Office Action and places the pending claims in condition for allowance. Should any issues remain to be discussed in this application, the undersigned may be reached by telephone. Please charge any fees due for consideration of this paper, including fees for extension of time, to the undersigned's Deposit Account No. 50-0206.

Respectfully submitted,

HUNTON & WILLIAMS LLP

Dated: 10/26/05

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Exhibit A

Lide, D. R. The CRC Handbook of Chemistry and Physics. CRC Press (2003).

CRC

HANDBOOK
of
CHEMISTRY
and
PHYSICS

DAVID R. LIDE
Editor-in-Chief

84th
EDITION
2003-2004

CRC PRESS

New data...
New journals...

Physical
Constants
of Organic
Compounds

Supplemental tables of
Physical Constants

Supplemental tables of
Properties of Polymers

Supplemental tables of
Properties of Inorganic
Compounds

Supplemental tables of
Properties of Organic
Compounds

Supplemental tables of
Properties of Polymers

Supplemental tables of
Properties of Inorganic
Compounds

Supplemental tables of
Properties of Organic
Compounds

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DISSOCIATION CONSTANTS OF ORGANIC ACIDS AND BASES (continued)

Mol. Form.	Name	Step	<i>t</i> °C	<i>pK_a</i>	Mol. Form.	Name	Step	<i>t</i> °C	<i>pK_a</i>
$C_5H_8O_2P_2$	1-Hydroxy-1,1-diphosphosuccane	1		1.35	C_3H_6NO	2-Methoxyethylamine		25	9.40
		2		2.87	C_3H_6NO	Trimethylamine oxide		20	4.65
		3		7.03	$C_3H_8N_2$	1,2-Propanediamine, (2)	1	25	9.82
		4		11.3			2	25	6.61
$C_3H_5O_2$	2-Propanoic acid		25	1.84	$C_3H_6N_2$	1,3-Propanediamine	1	25	10.55
C_3H_5NO	Oxazole		25	0.8			2	25	8.88
C_3H_5NO	Isoxazole		25	-2.0	$C_3H_6N_2O$	1,3-Diamino-2-propanol	1	20	9.69
$C_3H_5NO_2$	Cyanosuccinic acid		25	2.47			2	20	7.93
C_3H_5NS	Thiazole		25	2.52	$C_3H_6N_3$	1,2,3-Triaminopropane	1	20	9.59
$C_3H_5N_2O_2$	Cyanuric acid	1		6.88			2	30	7.95
		2		11.40	$C_3H_6PN_2O$	Fluconoxine			3.26
		3		13.5	$C_3H_6N_2$	Pyrazine		20	0.65
$C_3H_4N_2$	1 <i>H</i> -Pyrazole		25	2.49	$C_3H_6N_2$	Pyrimidine		20	1.23
$C_3H_4N_2$	Imidazole		25	6.99	$C_3H_6N_2$	Pyridazine		20	3.24
$C_3H_4N_2S$	2-Thiazolamine		20	5.36	$C_3H_6N_2O_2$	Uracil		25	9.45
C_3H_6O	Propargyl alcohol		25	13.6	$C_3H_6N_2O_2$	Bartinic acid		25	4.01
$C_3H_6O_2$	Acrylic acid		25	4.25	$C_3H_6N_2O_2$	Alloxanic acid		25	6.64
$C_3H_6O_2$	Pyruvic acid		25	2.39	$C_3H_6N_2O_2$	5-Nitropyrimidinamine		20	0.35
$C_3H_6O_4$	Maleic acid	1	25	2.85	$C_3H_6O_2$	2-Butynoic acid		25	2.62
		2	25	5.70	$C_3H_6O_4$	Maleic acid	1	25	1.92
$C_3H_6O_5$	Hydroxypyruedioic acid	1		2.42	$C_3H_6O_4$	Fumaric acid	1	25	6.23
		2		4.54			2	25	3.02
$C_3H_5BrO_2$	3-Bromo-2-propanoic acid		25	4.00	$C_3H_6O_5$	Oxaloacetic acid	1	25	2.25
$C_3H_5ClO_2$	2-Chloro-2-propanoic acid		25	2.83			2	25	4.37
$C_3H_5ClO_2$	3-Chloro-2-propanoic acid		25	3.98			3	25	13.03
$C_3H_5N_2$	3-Aminopropenonitrile		20	7.80	C_3H_6N	Pyrrole			3.8
$C_3H_5N_2$	1,3,5-Triazine-2,4,6-triamine		25	5.00	$C_3H_6NO_2$	Succinimide			9.62
C_3H_6O	Allyl alcohol		25	15.5	$C_3H_6N_2$	2-Pyrimidinamine		20	3.45
$C_3H_6O_2$	Propanoic acid		25	4.87	$C_3H_6N_2$	4-Pyrimidinamine		20	5.71
$C_3H_6O_2S$	(Methylthio)acetic acid		25	3.66	$C_3H_6N_2O$	Cysteine	1		4.60
$C_3H_6O_3$	Lactic acid		25	3.86			2		12.16
$C_3H_6O_3$	3-Hydroxypropanoic acid		25	4.51	$C_3H_6N_2O_2$	6-Methyl-1,2,4-triazine-3,5(2H,4H)-dione			7.6
$C_3H_6O_4$	Glycine acid		25	3.52	$C_3H_6N_2$	1-Methylimidazole		25	6.95
C_3H_6N	Allylamine		25	9.49	$C_3H_6N_2O_2$	Alantoin		25	8.96
C_3H_6N	Azetidine		25	11.29	$C_3H_6N_2O_2S_2$	Acetamolamide			7.2
C_3H_6NO	2-Propanone oxime		25	2.42	$C_3H_6O_2$	trans-Crotonic acid		25	4.69
$C_3H_6NO_2$	<i>L</i> -Alanine	1	25	2.34	$C_3H_6O_2$	3-Butenoic acid		25	4.34
		2	25	9.87	$C_3H_6O_2$	Cyclopropanecarboxylic acid		25	4.83
$C_3H_6NO_2$	β -Alanine	1	25	3.55	$C_3H_6O_2$	2-Oxobutanoic acid		25	2.50
		2	25	10.24	$C_3H_6O_2$	Acetoacetic acid		25	3.6
$C_3H_6NO_2$	Sarcosine	1	25	2.21	$C_3H_6O_4$	Succinic acid	1	25	4.21
$C_3H_6NO_2S$	<i>L</i> -Cysteine	2	25	10.1			2	25	5.64
		2	25	1.5	$C_3H_6O_4$	Methylmalonic acid	1	25	3.07
$C_3H_6NO_2S$		2	25	8.7			2	25	5.76
		3	25	10.2	$C_3H_6O_4$	Malic acid	1	25	3.40
$C_3H_6NO_2S$	<i>L</i> -Serine	1	25	2.19			2	25	5.11
		2	25	9.21	$C_3H_6O_4$	<i>D,L</i> -Tartaric acid	1	25	3.03
$C_3H_6NO_2S$	<i>D,L</i> -Cysteic acid	1	25	1.3			2	25	4.37
		2	25	1.9	$C_3H_6O_5$	<i>meso</i> -Tartaric acid	1	25	3.17
$C_3H_6NO_2O_2$	Glycocyamine		25	2.82			2	25	4.91
$C_3H_6O_2$	Ethylene glycol monomethyl ether		25	14.8	$C_3H_6O_5$	<i>L</i> -Tartaric acid	1	25	2.98
$C_3H_6O_3$	Glycogen		25	14.15			2	25	4.34
C_3H_6N	Propylamine		25	10.54	$C_3H_6O_5$	Dihydroxytartaric acid		25	1.92
C_3H_6N	Isopropylamine		25	10.63	$C_3H_6ClO_2$	2-Chlorobutanoic acid			2.86
C_3H_6N	Trimethylamine		25	9.80	$C_3H_6ClO_2$	3-Chlorobutanoic acid			4.05
					$C_3H_6ClO_2$	4-Chlorobutanoic acid			4.52

Exhibit B

Windholz, M. The Merck Index: An encyclopedia of chemicals and drugs. Merck & Co. (1989).

THE MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

ELEVENTH EDITION

Susan Budavari, Editor
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Published by
MERCK & CO., INC.
RAHWAY, N.J., U.S.A.

1989

HGG	human gamma globulin	I.U.P.A.C.	International Union of Pure and Applied Chemistry
BGH	hur	i.v.	intravenous
His	hist	Japan, Kokai	Japanese patent (unexamined)
HIV	hur	Japan, pat.	Japanese patent
HLA	hur	K	dissociation constant, equilibrium constant
HMG-CoA	3-h		degrees Kelvin
			kilobase
Houben	a G	K	kilocalorie(s)
	F	Kb	killer cell
Houben Weyl	Ho	kcal	kilograms)
		K cell	α -ketoglutarate
		Kg	keyhole limpet hemocyanin
HPLC	Organischen Chemie, a German collection of preparative methods in organic chemistry (Thieme)	KLH	prourokinase, kidney plasminogen activator
hr	high performance (pressure, power) liquid chromatography	KPA	liter
HSA	hour	J	levorotatory), the opposite of <i>d</i> , <i>g.v.</i>
Hse	human serum albumin	J-	<i>levo</i> (in configurational sense only), the opposite of <i>D</i> , <i>g.v.</i>
HSV	herpes simplex virus	L	lactose
HT	hydroxytryptamine (serotonin)	LAD	lymphocyte-activating determinant
HTLV	human T lymphotropic virus see also HIV	λ (lambda)	wavelength
Hyl	hydroxylysine	LATS	long acting thyroid stimulator
Hyp	hydroxyproline	lb	posure
<i>i</i>	optically inactive by internal compensation as <i>l</i> -inositol; arctic for <i>meso</i> -inosine	LC	Lethal Concentration; LC_{50} , a concentration which is lethal to 50% of the animals tested; liquid chromatography
I	I-region antigen	LCM	lymphocyte choriomeningitis
Ia	International Association of Cancer Registries	LD	Lethal Dose; LD_{50} , a dose which is lethal to 50% of the animals tested
IACR	International Agency for Research on Cancer	LDH	lactate dehydrogenase
IARC Monographs	IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man	LDL	low density lipoproteins
<i>ibid</i>	(<i>ibidem</i>) at the same place	Leu	leucine
I.C.C.	Interstate Commerce Commission	LH	luteinizing hormone (same as ICSH)
ICFA	incomplete Freund's adjuvant (same as FIA)	In	natural logarithm
ICSH	interstitial cell-stimulating hormone (same as LH)	LNPF	lymph node permeability factor (<i>loco citato</i>) in the place cited
<i>idem</i>	the same (author); plural: <i>eidem</i> , the same (authors)	loc. cit.	logarithm (common)
IDP	inosine diphosphate	log	limit of impurities
I.e.	(<i>idem</i>) that is	l.o.i.	lipopolysaccharide
IEF	isoelectric focusing	LPS	lysine
IF, IFN	interferon	Lys	met _n ; given after mass number signifies metastable isomer
i.g.	intragastric	m	<i>meta</i>
Ig	immunoglobulin	m-	molar (concentration)
I.G. Farben	Interessengemeinschaft der Farbenindustrie, Aktiengesellschaft- the German dye trust	M	monoclonal antibody
IGF-I	insulin like growth factor I	Mab, mAb	maximum allowable concentration
IL	interleukin	MAC	macrophage activating factor
Ile	isoleucine	MAF	monoamine oxidase
i.m.	intramuscular	MAO	monoamine oxidase inhibitor
IMP	inosine 5'-monophosphate (inosinic acid)	MAOI	mass spectrometry
incl	including	mass spec	maximum, maxima
incompat	incompatibility	max	myoglobin
INN	International Nonproprietary Name	Mb	oxymyoglobin
inorg	inorganic	MbO ₂	Manufacturing Chemists Association (U.S.A.)
insol	insoluble	M.C.A.	microgram
intern	internal	M _D	millicurie
Intl	International	mcg	molecular rotation $\frac{[\alpha]_D \times \text{mol wt}}{100}$
i.p.	intraperitoneal	mCi	malate dehydrogenase
IR	infrared	MdH	methyl CH ₃
Ir genes	immune response genes	Me	acetone
ISO	Internal Organization for Standardization	Me ₂ CO	methyl alcohol
isoin	isolation	MeOH	<i>Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry</i> (Longmans)
ITP	inosine triphosphate or idiopathic thrombocytopenic purpura	Mellor's	milli-equivalent (0.001 of an equivalent)
I.U.	international unit	mEq	methionine
I.U.C.	International Union of Chemistry	Met	<i>N</i> -formylmethionine

MethHb	methemoglobin	OA	ovalbumin
meV	millielectron volts	OAA	oxaloacetate
mfq, manuf	manufacturing	OD	optical density
mfr	manufacture	op. cit.	(<i>opere citato</i>) in the work cited
mg	milligram	org	organic
MHC	major histocompatibility complex	OSHA	Occupational Safety and Health
μ Ci	microcurie	Act	Act
μ g	microgram	OsM	osmolar
microcryst	microcrystalline	oz	ounce(s)
MIF	migration inhibition factor	P or p	concentration by weight (after optical rotations only)
min	minimum; also minute(s)	p, pp	page(s)
MIS	Mullerian inhibiting substance	p-	<i>para</i>
misc	miscible	P ₁	inorganic phosphate
mixt	mixture	Pa	pascal
ml	milliliter (cubic centimeter)	PABA	<i>p</i> -aminobenzoic acid
MLD	minimum lethal dose	PAF	platelet-activating factor
MLR	mixed lymphocyte reaction	PAGE	polyacrylamide gel electrophoresis
mm	millimeter	passim	here and there, scattered
μ μ	micrometer(s), nanometer	pat.	patent
mol wt	nanometer(s), eight	PB report	Publication Board Report (United States Department of Commerce, Scientific and Industrial Reports)
Monash.	Monographs for Chemists	PCA	passive cutaneous anaphylaxis
mp	melting point: melts, melting at, when followed by a figure	PCT	Patent Co-operation Treaty
M _r	denoting temperature	PEP	phosphoenopyruvate
ms	relative molecular mass	petr. petrol	petroleum
MS	meso- (internally compensated)	PFC	plaque-forming cell
MSH	mass spectrometry	3PG	3-phosphoglycerate
n	melanocyte-stimulating hormone (melanotropin)	PG	prostaglandin
	index of refraction (η_d^2 for 20° and sodium light); normal, as <i>n</i> - propyl	PGA	pteroylglutamic acid (folic acid)
N	normal (equivalents per liter, as applied to concentration): nitrogen (as <i>N</i> -methyl- pyridine)	PGP	3-phosphoglycerol phosphate
	<i>N</i> -acetylneurameric acid	pH	acid-base scale; log of reciprocal of hydrogen ion concentration
NAcneu	nicotinamide adenine dinucleotide (reduced form)	PHA	phytohemagglutinin
NAD ⁺ (NADH)	nicotinamide adenine dinucleotide (phosphoreduced form)	Phe	phenylalanine
NADP ⁺ (NADPH)	nicotinamide adenine dinucleotide phosphate (reduced form)	physiol	physiology
NANSAIDS	nonsteroidal anti- inflammatory drugs	pK	log of the reciprocal of the dissociation constant, 1/ $\log K$ ↗
NBS	National Bureau of Standards	PKU	phenylketonuria
NCTC	National Collection of Type Cultures	PMN	polymorphonuclear leukocyte
NDP	nucleoside 5'-phosphate	PP _i	inorganic pyrophosphate
Neth. pat.		ppm	parts per million
Appl.	Netherlands patent application	ppi(g)	precipitate, precipitating
N.F.	National Formulary	ppi(d)	precipitated
ng	nanogram (10^{-9} grams)	PQ	plastoquinone
NGF	nerve growth factor	P _r	propyl (normal)
NIOSH	National Institute for Occupational Safety and Health	prep'd	prepared
nm	nanometers	prep'n	preparation
NMN ⁺ (NMNH)	nicotinamide mononucleotide (reduced form)	press.	pressure
NMP	nucleoside 5'-monophosphate	Pro	proline
NMR	nuclear magnetic resonance	PRPP	5-phosphoribosyl 1-pyrophosphate
N.N.D.	New and Nonofficial Drugs	ψ (psi)	pseudo
	(Lippincott, 1959-1964)	pt	point
N.N.R.	New and Nonofficial Remedies	PTH	parathyroid hormone
	(Lippincott, 1933-1958)	Q	coenzyme Q (ubiquinone)
no.	number	q.q.v.	(<i>quae vide</i>) which see, plural
nor-	(<i>Nitrogen ohne Radikal</i>) a prefix indicating a parent compound (no longer limited to nitrogenous compounds)	q.v.	(<i>quod vide</i>) which see
NRDC	National Research Development Corporation	R	"roentgen" unit of radiation. That quantity of x or gamma radiation which produces one esu of charge in one cubic centimeter of air under standard conditions, i.e., the associated corpuscular emission per 0.001293 g of air (1 cc at 0° and 760 mm) produces, in air, ions carrying one esu
NSAID	nonsteroidal anti-inflammatory drugs	(R)	racemic
NSC	National Service Center		alkyl, univalent hydrocarbon radical (or hydrogen)
NTP	nucleoside 5'-triphosphate		rectus (right). Also a term of art for the spatial arrangement about an asymmetric carbon when the observed order of decreasing priority of the groups is clockwise
σ -	ortho		
O	denoting attachment to oxygen, as in <i>O</i> -acetylhydroxylamine		

D-Lactic Acid

35,000. Two types of subunits are distinguishable: M (muscle) type and H (heart) type. Lactic dehydrogenases of heart and muscle are mainly H_4 and M_4 ; all other possible hybrids have been found in various tissues. Activities of lactate dehydrogenase activity have been found in myocardial infarction, hepatocellular necrosis, metastatic carcinoma, diabetic ketosis, sickle cell anemia, malignant lymphoma, infectious mononucleosis, and cerebral infarction: Standinger *et al.*, *J. Am. Med. Assoc.* 182, 1099 (1962). Comprehensive review: Everse, Kaplan, *Advanc. Enzymol. Relat. Areas Mol. Biol.* 37, 193 (1973); Holbrook *et al.*, in *The Enzymes*, vol. XI (part A), P. D. Boyer, Ed. (Academic Press, New York, 1973) pp 191-292.

USE: In the determination of pyruvate (used in conjunction with reduced coenzyme). In the diagnosis of myocardial infarction and leukemia.

5214. D-Lactic Acid. (*D*)-2-Hydroxypropanoic acid; *D*-(-)-lactic acid; levatoractic acid; *L*-lactic acid; *D*-Milchsaure (German); $C_3H_4O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Obtained by resolution of *L*-lactic acid: Purdie, Walker, *J. Chem. Soc.* 61, 754 (1892); Borsig *et al.*, *J. Biol. Chem.* 102, 449 (1933). Convenient laboratory prep from glucose using *Lactobacillus leichmannii*: Brin, *Biochem. Prepr.* 3, 61 (1953).



Crystals from ether + isopropyl ether, mp 52.5°. $[\alpha]_D^{25} -2.6^\circ$ ($c = 8$). $\rho K = 3.83$. Sol in water, alcohol, acetone, ether, glycerol. Practically insol in chloroform.

Forms salts with many metals. Most of these salts are decolorizing.

Zinc *D*-lactate, $Zn(C_3H_4O_3)_2 \cdot 2H_2O$, crystals, $[\alpha]_D^{25} +8.18^\circ$ ($c = 2.5$).

5215. DL-Lactic Acid. 2-Hydroxypropanoic acid; racemic acid; ordinary lactic acid; α -hydroxypropanoic acid; Milchsaure (German); Lactovagan; Tonsillosan (Lüsing). $C_3H_4O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Occurs in sour milk as a result of lactic acid bacteria; also found in molasses due to the conversion of sugars, in apples and other fruits, tomato juice, beer, wine, opium, ergot, foxglove, and several higher plants, especially during germination. Lactic acid is prep technically by "Lacto-fermentation" of carbohydrate such as glucose, sucrose, lactose with *Bacillus acidilacti* or related organisms, such as *Lactobacillus delbrueckii*, *L. bulgaricus* etc. The fermentation is carried out at relatively high temps. Produced commercially by fermentation of whey, cornstarch, potatoes, molasses. Review on the production of lactic acid by fermentation: S. C. Prescott, *Ind. Eng. Chem. Microbiology* (McGraw-Hill, New York, 3rd ed., 1959) pp 304-331. Chem. preps from acetaldehyde and CO_2 in oil at 130-200°: Loder, *U.S. pat. 2,268,945* (1943) to J. Pont; by hydrolysis of hexoses with NaOH : Lock, *U.S. pat. 2,382,889* (1943). Preps of crystalline lactic acid: Borsig *et al.*, *J. Biol. Chem.* 102, 449 (1933). Toxicity data: Smyth *et al.*, *J. Ind. Hyg. Toxicol.* 23, 259 (1941).



Crystals, mp 16.8°. $\text{bp}_{14-15} 122^\circ$; bp_{41-4} 82-85°. K at 25° 1.38×10^{-4} . Heat of combustion at constant pressure 3615 cal/kg. Volatile with steam. Sol in water, alc., furfurol; less sol in ether. Practically insol in chloroform, petr ether, carbon disulfide. Pharto. form: Oxidizing agents, iodides HNO_3 -aluminiun. LD_{50} orally in rats: 3.73 g/kg (Smyth).

Barium salt, $\text{C}_3H_4BaO}_6$, *barium lactate*. Powder. Poisonous. Sol in water, dil alcohol.

Copper salt dihydrate, $\text{C}_3H_4CuO}_2 \cdot 2H_2O$, *cupric lactate*.

Green to blue crystals. Readily sol in water; practically insol in alcohol.

USE: In dyeing baths, as mordant in printing woods, goods, solvents for water-insoluble dyes (alcohol-soluble indoline, nigrosine, spirit-blue); reducing chalcogenates in mordanting wool, mussel cheese, confectionery; oxidant in mordanting for acidulating worts in brewing, for removing *Chloroform*, bacterium in must of yeast; dehairing, plumping, and descaling hides; solvent for cellulose formic flux for soft solder; many lactates which are used in food products, in medicine, and as solvents; plasticizer, catalyst in the casting of phenolaldehyde resins. *Caution:* Caustic in concentrated solns.

THERAP CAT: Acidulant.

THERAP CAT VET: Has been used as a caustic, and in dilute solutions to irrigate tissues; as an intestinal antiseptic and antidiarrheal.

5216. L-Lactic Acid. (*S*)-2-Hydroxypropanoic acid; *L*-(-)-lactic acid; dextrotoratory lactic acid; *d*-lactic acid; *L*-lactic acid; paralactic acid; Fleischmilchsäure (*L*-Milchsaure); $C_3H_4O_3$; mol wt 90.08. C 40.00%, H 6.71%, O 53.29%. Occurs in small quantities in the blood and muscle fluid of man and animals. The lactic acid concs increases in muscle and blood after vigorous activity. *L*(+)-Lactic acid is also present in liver, kidney, pituitary gland, human amniotic fluid, and other organs and body fluids. Obtained by resolution of *D*-lactic acid: Purdie, Walker, *J. Chem. Soc.* 61, 754 (1892); Borsig *et al.*, *J. Biol. Chem.* 102, 449 (1933). Convenient laboratory prep from glucose by fermentation by *Lactobacillus delbrueckii*: Brin, *Biochem. Prepr.* 3, 61 (1953). Preps from hexoses using *R. desmodioides*: Ashton, Davies, *Ind. Eng. Chem.* 34, 1522 (1942). Monograph: M. Brin, R. H. Dunlop, "Chemistry and Metabolism of *L*- and *D*-Lactic Acids", *Ann. N.Y. Acad. Sci.* vol. 119, art. 3, 851-1165 (1963).



Crystals from acetic acid or chloroform, mp 53°. $[\alpha]_D^{25} +2.6^\circ$ ($c = 2.5$). $\rho K = 25.7$. Forms salts with many metals. The salts are more sol in water than the salts of the racemic acid. Most of these salts are levatory.

Zinc *L*(+)-lactic dihydrate, $Zn(C_3H_4O_3)_2 \cdot 2H_2O$, prism. $[\alpha]_D^{25} -8.2^\circ$ ($c = 2.5$ in water).

5217. Lactic Acid Lactate. 2-Hydroxypropanoic acid 1-carboxyethyl ester, 2-(methylacetoxy)propanoic acid; 2-(2-hydroxypropanoyloxy)propanoic acid. $C_5H_8O_4$; mol wt 162.14. C 44.44%, H 6.22%, O 59.34%. Prep'd by heating lactic acid at 120° for 10 hours: Dietz, Krug, *Ber. 88*, 1307 (1925).



Pale yellow, clear, odorless oil. Sol in water and in the usual organic solvents.

Methyl ester, $C_5H_8O_3$. Prep'd: Clasborn, *U.S. pat. 2,371,281* (1945) to the people of the U.S. $\text{bp}_{14-15} 107^\circ$; $\text{ng} 1,431$.

USE: The methyl ester as a solvent or plasticizer.

5218. Lactobacillic Acid. 2-Hexylpropanoic acid; 11,12-methylenesuccinic acid; phytomonic acid. $C_9H_{16}O_4$; mol wt 296.4. C 73.97%, H 12.24%, O 10.79%. A lipid constituent of various microorganisms. Isolated from *Lactobacillus arabinosus*: K. Hofmann, R. A. L. Tausig, *J. Am. Chem. Soc.* 72, 4328 (1950); from *Agrobacterium* acid: K. Hofmann, P. Tausig, *J. Biol. Chem.* 213, 425 (1955). Structure: K. Hofmann *et al.*, *J. Am. Chem. Soc.* 80, 5717 (1958). Abs config: J. F. Tocanne, *Tetrahedron* 28, 363 (1972).